Notes on some Parasitic Protists.

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With Plate 6.

During the last few years the Bacteria and their allies have engaged the attention of so many workers, have caused so much controversy, and have proved to be of such great interest—phylogenetically and otherwise—that I believe any observations, however small, which would supplement our knowledge of their cytology or life-history, are much to be desired. With this conviction I offer these few imperfect notes on some of the more interesting protists which have come under my observation from time to time.

I. Bacillus flexilis, n. sp. (Pl. 6, figs. 1—19.)

More than five years have now elapsed since the appearance of Schaudinn's epoch-making paper on Bacillus bütschlii, a parasite of the common cockroach, P. orientalis. Yet, apart from Schaudinn's own further researches on the Bacteria, no similar life-history has since been recorded. However, I can now record the existence of an organism which in many respects exhibits a remarkable similarity to B. bütschlii.

I propose to name this organism Bacillus flexilis, n. sp., although I must point out that I do not believe it should

really be placed in the genus Bacillus at all. But B. bütschlii has now remained in this genus for some time, and I think my organism must go where it goes. Both forms will probably have to be removed—perhaps, as Schaudinn suggested, to the genus Dispora, Kern. The large size of these organisms, their life-history and formation of two spores, all distinguish them from the ordinary Bacilli. The specific name is given on account of what is, at first sight, the most striking characteristic of the organism—namely, its flexibility.

B. flexilis is found in the large intestine of frogs and toads —Rana temporaria, L., and Bufo vulgaris, L. It is, like B. bütschlii, of rare occurrence; I have found it in only 3—4 per cent. of animals examined, and on only one occasion in large numbers. I have also found a similar, though longer and more slender organism, in the common newt.

In size the organism varies considerably, and may be any length from 15 μ to 35 μ. On one occasion I found a specimen (undivided) which had attained a length of 39·5 μ. An average length is about 25 μ. The breadth is from 2—3 μ. It will be seen, therefore, that, although a giant among Bacteria, this protist is only about half the size of B. bütschlii.

The shape is that of a long rod, with the ends bluntly pointed rather than rounded. The protoplasm is finely granular and somewhat alveolar in the living organism, but by no means so distinctly "honeycombed" as in B. bütschlii (see Pl. 6, fig. 1). A strongly-developed outer layer can be seen on careful focussing (fig. 1) consisting of the pellicle and perhaps also of an ectoplasmic sheath. The organism is clad all over with very fine cilia ("flagella" of the bacteriologist), which can be demonstrated in stained preparations only (see fig. 9).

A large number of granules inside the organism are found to stain with nuclear stains. I am inclined to regard these as being composed of chromatin, and the organism, therefore,

according to my view, contains a "diffuse nucleus" in the form of a chromidial system.[1]

The movements of B. flexilis are very characteristic. By means of its "flagella" it swims rapidly from place to place in either direction. In addition to these locomotory movements, intrinsic movements can be observed. They consist, for the most part, in a lateral bending, and appear to be largely passive, determined by the disposition of the débris through which the organism is making its way. Owing to these movements, the organism presents a curved, S-shaped or sometimes somewhat spiral appearance. As it swims about its ends often oscillate slowly from side to side, pendulum-wise.

In being so remarkably supple, this Bacillus differs from all others. In fact, rigidity has sometimes been used as a criterion for judging the bacterial or protozoan nature of an

[1] It is perhaps necessary to say a few words about these granules in Bacteria. Colourable granules have been long known to occur in bacterial cells. They were described as "metachromic bodies" by Babès, "red granules" by Bütschli, "sporogenic granules" by Ernst, etc., etc. They have been variously interpreted: by some (Bütschli, Schewiakoff, etc.) as chromatin, by others (Fischer, Migula, etc.) as metabolic products, by others (Meyer, Grimme, etc.) as "volutine," by others still (e.g. Podwyssotzski) as products of degeneration, by others again (e.g. von Behring) as toxigen granules, and so on and so on. All views have, in consequence, been maintained regarding the cytology of Bacteria—from that which regards them as all cytoplasm to that which regards them as all nucleus. The latter view has recently been upheld by Růžička, who believes Bacteria to consist entirely of nuclein, on account of their resistance to artificial gastric juice. Bütschli first put forward this view, and it was held by Klebs, Hüppe, and many others subsequently. On the other hand, many writers, since Schottelius in 1888, maintain that a true nucleus exists in Bacteria. Such a view has been held by Vejdowský, Mencl, A. Meyer, etc., etc., whose work seems to prove conclusively that, in some cases at least, a distinct nucleus is present.

It appears highly probable that more than one kind of granule exists in the bacterial cell, and that in many cases the granules are in part chromatin. Schaudinn, Guilliermond, and others adopt this view. The "metachromic bodies" are probably reserve material. Meyer and Guilliermond have shown that they exist side by side with a true nucleus in the Ascomycetes—an observation of much interest.

organism—for instance, in the case of the spirilla and spirochæts. In speaking of Bacillus bütschlii, Schaudinn says, "Biegung des Stäbchens findet man äusserst selten;" so that in this respect it must differ markedly from my Bacillus. The only other organisms which appear to be in any way comparable, are the remarkable forms included in the Myxobacteriaceæ of Thaxter. But here, although the rods are flexible, they are not motile in the same manner, for they progress by creeping movements on a resistant surface. (See Baur's description.)

Division is effected in B. flexilis by a simple constriction into two, as in B. sporonema, Schaudinn. In the method of division, therefore, another point of difference between my organism and B. bütschlii is seen: for in the latter, division takes place by septation. I have watched division in the living organism, and have stained permanent preparations at various stages, but I think it is not necessary to enlarge upon a process which has been already described in so many bacteria (see Plate 6, fig. 10).

The most interesting phenomena are those connected with spore formation. I will describe these first, and comment on them afterwards. Unfortunately, I have not been able to follow the whole process from beginning to end in one and the same living organism: for, at the end of a period varying from three to five hours, my protists always died in hanging drop or coverslip preparations. But I have been able to watch spore-formation in different organisms in consecutive stages, and I believe, the following is a substantially correct account of the normal course of events. The observations have all been confirmed on stained material.

The organisms which are about to form spores are of considerable length—usually about 30 μ to 35 μ—and are filled with distinct granules, which stain a purple-red with Giemsa. After swimming about in this condition for some time, the rod begins to show a slight constriction at its middle, and appears to be about to divide (fig. 10). Division is not completed, however, and after the lapse of some time, it can be

seen that the constriction, which at one period almost severed the rod into two daughter rods, is slowly disappearing (fig. 11). The granules at this period are very distinct and numerous, but I have never succeeded in convincing myself that any streaming movements were taking place, on account of the active movements of the organism. It is probable, however, that such granule-streaming does occur, as in B. bütschlii, where Schaudinn was able to watch it with great precision. As the constriction disappears, the granules begin to rearrange themselves in the cell, but so slowly that their movements cannot be noticed whilst the organism as a whole remains motile. At the end of some hours, the granules are seen to be disposed in a somewhat irregular spiral (figs. 2 and 13). This spiral is quite distinctly visible in the living organism. It reminds one of the figures given by Swellengrebel of B. maximus buccalis. As in this case, the spiral appears to be formed of chromatin, the chromidia having rearranged themselves in this form.

I do not know how long the organism remains in this condition, but it is probably for several hours. At the end of this time, small, clear areas can be seen at either end of the spiral (fig. 4). These are the spore rudiments, and at first they look like vacuoles. It can be seen in the stained preparations that they are formed by the concentration of the chromatin at these spots (fig. 14). These chromatic areas increase in size (figs. 5, 15), and as they do so the intermediate part of the spiral begins to break down (figs. 14, 15). When a later stage is reached, the spores appear as dense, ovoid masses of chromatin, and the intermediate chromatin breaks up into fine granules (fig. 16). After this, a clear area makes its appearance round the spore (fig. 6). This is the first appearance of the spore membrane. Its presence is easily seen in the living organism, and is strikingly displayed in Giemsa preparations: for the spores, which have hitherto been coloured red, now appear deep blue (fig. 17). The spore membrane gradually grows hard and resistant, and as its induration increases, it stains less and less (fig. 18). Finally,

the spores become exceedingly hard and refringent (fig. 7) and refuse to stain in the slightest degree.

Whilst the spore membrane has been forming, the intermediate part of the spiral has broken down completely, and as it degenerates, the protoplasm changes its staining reactions. At the beginning, the spiral was red, in a bluish cytoplasm (figs. 13, 14, 16). As it degenerates, and diffuses through the protoplasm, it imparts to it a purplish (fig. 17), and finally a pink tinge (fig. 18). The spores, which at first occupied the ends of the cell, now move towards one another somewhat, so that little polar caps of protoplasm are left at the extremities of the rod. In contradistinction to the rest of the protoplasm, these caps stain blue with Giemsa (fig. 18). The rods with their two terminal spores present a very characteristic appearance at this stage (figs. 3, 18), for they remain actively motile for some time longer, sometimes for several hours. In the end, they come to rest, and the protoplasm degenerates (fig. 19), so that the two fully-formed spores alone remain, connected by the dead and broken remains of the organism.

The fully formed spores, like those of B. bütschlii, vary somewhat in shape, and show a small cap-like eminence at one end (fig. 8). This, in B. bütschlii, is the pole at which germination occurs. I regret that I have not been able to watch the germination of the spores in B. flexilis. In size, the spores are about 5 $\mu \times 2 \mu$, and hence cannot be easily mistaken for any other spores found in the same locality.

I should point out that the figures of stained specimens have all been drawn from Giemsa preparations in which drying has taken place between staining and mounting. They are all, therefore, somewhat flattened out, and so appear broader than they really are (see especially figs. 9, 14, and 17). This flattening of the organisms serves, however, to show their structure more clearly. And although the method is not to be recommended for the most accurate work, it gives very pretty results. I have used the method only in conjunction with others (see Appendix), but the results have

been essentially the same in all cases, and merely confirm what can be seen in the living organism.

I have encountered very many degenerate and plasmolysed forms of different kinds. Almost all of these have been nearly identical with similar conditions figured by Schaudinn in B. bütschlii. Indeed, his figures might easily stand for B. flexilis. I have found vacuolated forms, forms in which abnormal division into three is occurring, forms in which an abnormal formation of a single spore has taken or is taking place, etc., etc. I do not think it is worth while to describe these again in detail, but I may mention that I have also found involution forms which reached the enormous length of nearly 200 μ, incompletely divided into as many as six cells, the whole chain writhing fitfully for hours, but owing to its unwieldy length unable to make any progress.

Spore-formation in Bacillus flexilis resembles in a remarkable manner that of Bacillus bütschlii, as anyone who will take the trouble to compare my figures with those of Schaudinn can see. In both organisms the incomplete division into two daughter-cells is only temporary; it is followed by a re-arrangement of the granules into a spiral filament, and by the heaping up of the granules at the ends, two spores arise in a closely similar manner—a part of the spiral degenerating.

Schaudinn, who discovered this remarkable process, looked upon it as a kind of primitive conjugation between daughter-cells, comparable to that which occurs in Actinosphærium (R. Hertwig), and—as he himself showed later—in Entamœba coli. He was also able to demonstrate the existence of a similar phenomenon in Bacillus sporonema, Schaudinn. I think the evidence is strongly in favour of such an interpretation.

A striking analogy exists in the case of the yeasts, which have yielded such interesting results to recent investigators. It is now conclusively proved that in some genera—e. g. Schizosaccharomyces and Zygosaccharomyces—conjugation, followed by sporulation, takes place between

daughter-cells which have not separated. These observations were made quite independently by Barker, Guilliermond, and others. A comparable process is also to be seen in the Algæ—e. g. Spirogyra—where adjacent cells may sometimes conjugate and form a zygospore. And there are further analogies to be found in the autogamy of the Protozoa (Bodo, Trichomastix, etc.), and the curious form of parthenogenesis sometimes seen in the Metazoa—for instance, in Artemia, according to the well-known description of Brauer.

The breaking up of the intermediate portion of the spiral may be regarded as a nuclear reduction—though it has yet to be proved that the filament really consists of chromatin. Similar filaments, consisting very probably of chromatin, occur in allied organisms, for example, in B. maximus buccalis (Swellengrebel), in Spirochæta balbianii (Perrin), and in Spirillum giganteum (Swellengrebel) (cf. also B. spirogyra, infra).

II. Bacillus spirogyra, n. sp. (Pl. 6, figs. 20*a*, *b*, *c*.)

I have found this Bacillus on several occasions in the rectum of frogs and toads. In the living condition it is an actively motile rod, of high refractivity. It shows no internal structure until stained—a circumstance which I believe to be due chiefly to the great thickness of its pellicle. In length the organism measures about 8—11 μ, and in breadth about 1·5—2 μ. It is, therefore, a bacillus of considerable size.

Upon staining the organism some additional points in its morphology can be made out. The very thick pellicle stains pink with Giemsa, and inside the organism a spiral filament is seen, which is stained like chromatin. That is to say, it becomes red or purple with Giemsa, black with iron-hæmatoxylin, etc. I believe it to be of a nuclear nature. It is reminiscent of the spiral structure described by Swellengrebel in B. maximus buccalis (see Pl. 6, fig. 20*a*).

The method of division is by septation, as in B. bütschlii. Before division the nuclear spiral becomes more coiled, and

very distinct. In the middle of the cell which is about to divide a transverse septum makes its appearance, staining strongly with Giemsa or iron-hæmatoxylin. The ends of the organism also stain darkly at all stages of its life; this staining of the ends and of the septum being a common condition in bacteria. At this stage the organism has the appearance shown in fig. 20*b*. Later the septum splits into two, and its halves then give rise to the ends of the daughter-cells (fig. 20*c*). These then separate.

Although this Bacillus resembles B. maximus buccalis in possessing a spirally disposed nuclear filament, it differs from it in its method of division. For in the latter a longitudinal splitting of the filament appears to take place, whereas in the former, as I have just shown, a transverse division can be seen. Swellengrebel's very careful micro-chemical investigation of B. maximus buccalis renders it highly probable that the filament consists of a substance which, if not chromatin, is at least very closely allied to it. In my Bacillus I have seen no separation of "chromosomes" and achromatic substance during division.

As this Bacillus does not appear to have been described before, I have named it Bacillus spirogyra, n. sp., from the fanciful resemblance which it bears to a Spirogyra cell with its contained chloroplast.

I have not yet succeeded in tracing out the rest of its life-history.

III. Spirillum monospora, n. sp. (Pl. 6, figs. 21*a*, *b*, *c*, *d*.)

A large species of Spirillum is not unfrequently found in the large intestine of frogs and toads. As I can find no record of it in the work of other investigators, I have called it Sp. monospora, n. sp., from the fact that it forms a single large terminal spore.

This Spirillum is usually S-shaped. Its dimensions are ca. 6—8 μ × ca. 1·5 μ. It is actively motile, and can be seen (in deeply stained iron-hæmatoxylin preparations) to possess

a flagellum (or bunch of flagella?) at either end. No simple nucleus, such as Kunstler and Gineste have described in Sp. periplaneticum, K. and G., can be made out; nor can a nuclear spiral, like that which Swellengrebel observed in Sp. giganteum, be seen. The only contents which stain consist of a number of granules, which recall the figures of Sp. volutans given by Bütschli. In this case again I can merely say that these granules stain in a manner which causes me to believe that they are chromatin (see Pl. 6, fig. 21*a*).

Transverse division takes place in the manner usually seen in the Spirilla. It will not, therefore, be necessary to describe the process. I may say, however, that I have not been able to find any specimens in which an unequivocal division of the nuclear granules is taking place.

Spore formation proceeds in the following manner. The nuclear granules, which in the vegetative stages are scattered throughout the cell, begin to aggregate at one end. A darkly staining, nucleus-like mass is thus produced (fig. 21*b*). A spore membrane appears round this, and, as it hardens, its staining capacity is gradually lost, so that finally an unstained, highly refringent spore becomes visible at the end of the organism (fig. 21*c*). Only a part of the granules enter into the formation of the spore. The remainder gradually degenerate, and are possibly in part volutine and other non-nuclear substances. Certainly a different intensity of coloration can often be noticed in the granules during even the vegetative stages of existence. Ultimately the whole of the cell-remains without the spore break down, so that the single spore is left with more or less of the dead cell attached (fig. 21*d*). Germination from the spore I have not observed.

Spore formation has been described previously in several Spirilla, e.g. in Sp. sporiferum, Mig., and in Sp. endoparagogicum, Sorokin. The details of the process are not known, however. Babès also found a Spirillum which sporulates, and he has figured it with deeply-stained masses at the ends during this process—resembling somewhat the

single terminal mass of nuclear granules in the spore formation of Sp. monospora.

Spirillum monospora remains actively motile until the spore is fully formed. It then presents a very characteristic appearance, as it swims about bearing its glistening, terminal spore.

IV. Spirochæta bufonis, n. sp. (Pl. 6, figs. 22*a*, *b*.)

I have little to say regarding this organism beyond recording its presence. It is very rare, so far as my experience goes. I have found it but once, in the rectum of Bufo vulgaris, L., and then in only very small numbers. It measures about 8—10 μ in length by about 1·5 μ in breadth. There are very few turns to the spiral, which displays some irregularities owing to the flexibility of the organism. The average distance between the turns is about 3 μ.

An undulating membrane or periplastic sheath can usually be seen extending from one end to the other. The spirochæt resembles S. buccalis, Cohn., but I have never seen terminal flagella, which are described in this species. The organism is very actively motile.

No internal structure has been satisfactorily demonstrated. A granular appearance of the protoplasm is usually to be seen, somewhat resembling that figured by Jaffé in S. culicis —another gut-inhabiting spirochæt, from Culex larvæ.

V. Treponema (?), sp. (Pl. 6, fig. 23*a*, *b*, *c*, *d*.)

An organism which appears to belong to the genus Treponema, Schaud., has been found by me on two occasions in the large intestine of the toad, B. vulgaris, L. Both animals were captured in the same locality, and contained the parasite in large numbers.

This organism is more difficult to observe in the living state than any other which I have ever encountered. It is

very active, and but feebly refractive. Its length varies from ca. 5—12 μ, whilst its breadth is exceedingly hard to gauge. From measurements of stained preparations, I judge it to be approximately 0·2 μ. The protist is flexible and spirally twisted, the distance between the turns of the spiral being about 2 μ. Some thicker forms are occasionally to be seen (fig. 23*a*), and others of an extraordinary slenderness (fig. 23*b*). In others (23*c*) the ends appear to taper into an exceedingly delicate filament, possibly a flagellum.

I have stained this organism with Heidenhain's iron-hæmatoxylin, and also with Giemsa. With the latter it stains a pale pink.

This protist bears a remarkable likeness to Treponema pallidum, Schaud., first described by Schaudinn, and since observed by many other workers, in syphilitic lesions. Whether these two forms are really related or not can only be decided by a knowledge of their life-histories. And unfortunately, despite the work of Krzysztalowicz and Siedlecki, and of many others, there is but little of the life-history of Schaudinn's organism known with certainty. I think it therefore premature to name this parasite from the toad.

Of its life-history I know nothing. Some of the longer forms show a thin place towards the middle, which might be due to division—either transverse or possibly the end of longitudinal. Even in the spirochæts—e. g. in S. balbianii—the method of division is disputed. For, although Perrin is assured that it is longitudinal, Swellengrebel believes it to be transverse, and regards longitudinal division as unproved. The appearances figured in T. pallidum by Schaudinn and many others certainly suggest that in this organism at least a longitudinal splitting is the rule.

I have purposely avoided discussing whether the spirochæts, etc., are Protozoa or Bacteria. For it seems to me fruitless to discuss the matter in our present state of ignorance. Indeed, those very characters which are taken by Schaudinn, Hoffmann and Prowazek and others to indicate their Protozoan affinities, are said by Swellengrebel to show

their relation with the Bacteria. I prefer, therefore, to employ the non-committal name of "protist."

Appendix.

On the Methods Employed in the Examination of Bacteria and Allied Organisms.

The methods usually employed by the bacteriologist for purposes of medical diagnosis are far too crude for the cytologist. It is not likely that a dried, plasmolysed, and flame-fixed mummy of a microbe can furnish much information regarding its structure during life. And, again, too much weight should not be given to observations made upon organisms grown in artificial and abnormal culture media. Such media are of the greatest value in the hands of the breeder of pathogenic Bacteria, but the development of the organisms in them cannot be unreservedly accepted as their normal life-history. The protistologist who would investigate the real ways of life of Bacteria must study them in their native medium, in the living state, and in carefully fixed and stained preparations.

Endoparasitic Bacteria usually survive but a short time in hanging drop preparations. They are much more suitably treated by being rapidly placed on a slide, covered with a coverslip with wax feet, and rapidly waxed round the edge with a small candle. In such preparations, if quickly and carefully made, they will live for a long time. They are best examined by means of a water-immersion apochromatic objective.

Intravitam staining is often very serviceable. The stains which have generally proved most useful are methylene blue, neutral red, and Brillantkresylblau.

All of these stains impart a more or less red colour to the metachromic granules during life.

In order to make good permanent preparations it is often necessary to dilute the medium in which the bacteria live.

Ordinary physiological salt solution usually makes the resulting film too watery to fix in a proper manner, but I have found that salt solution containing about 15 per cent. of egg albumen answers admirably. The Bacteria from the large intestine of Amphibia live in this medium for a long time, and very good moist film preparations can be made. Swellengrebel uses a solution of gelatine for the same purpose, and has been successful with Bacteria and yeasts.

The moist films made in this way should be as thin as possible and fixed immediately. For this purpose I know of nothing better than Schaudinn's sublimate-alcohol (2 : 1), used hot. Staining is best accomplished after this treatment by Heidenhain's iron-hæmatoxylin. Very good permanent preparations can be made in this way. I do not find it necessary to wash out the sublimate with alcohol containing iodine, as is usually recommended.

Good results may also be achieved by fixation with osmic vapour or in 1 per cent. osmic acid. And the method of fixation over the vapour from osmic and acetic acids (Plimmer, 'Proc. Roy. Soc.,' B., lxxix, 1907) gives good preparations.

A very good and simple way of fixing is that used by Swellengrebel. A small drop of culture fluid is spread out on a coverslip with a small drop of formalin solution (I use Schering's 40 per cent. formaldehyde) and allowed to dry. I find it best to harden the film in absolute alcohol for about fifteen minutes afterwards. One per cent. osmic acid can be used in the same manner as formalin. After such fixation Heidenhain or Giemsa may be used. Both give excellent results. Giemsa is not so successful after sublimate fixation.

Although drying before fixing is not to be recommended, it sometimes gives quite good results when followed by alcohol fixation and Giemsa—the method used by Perrin for Spirochæta balbianii. Fixation of moist films in absolute alcohol has often proved successful, but it not uncommonly causes plasmolysis.

I have used the following method of staining with Giemsa without drying at any period in the process, and have found

it quite successful :—Expose coverslip (carefully cleaned) to vapour from 1 per cent. osmic acid thirty seconds; spread out small drop of culture on exposed surface, and hold in vapour thirty seconds to one minute; harden in absolute alcohol ten to fifteen minutes; stain in Giemsa (Grübler, 1 drop to every c.c. of water) for twenty minutes; wash in water; dip rapidly into absolute alcohol; take coverslip quickly through three changes of xylol and alcohol (equal parts) into pure xylol; when quite clear mount in cedar oil. I have obtained equally good results with formalin-fixed films treated in this way. And I have also been able to make successful preparations in this manner—without drying—using as stains Löffler's methylene blue and corbol-fuchsin. Dehydration may also be effected without decolorising by using acetone (puriss. acid-free) instead of alcohol (Schridde, C.B. allg. Path. u. Anat., xvi, 1905). I have not been very successful with this method, but I believe with a little modification it could be made very effective.

Giemsa preparations should be mounted in cedar oil or neutral Canada balsam. These preparations are usually the prettiest, but I think iron-hæmatoxylin is more accurate.

Many other methods may, of course, be used in studying Bacteria. I have merely given those which I have myself found useful, in the hope that they may be of some use to others.

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5th September, 1907.

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EXPLANATION OF PLATE 6,

Illustrating Mr. C. Clifford Dobell's paper on "Notes on some Parasitic Protists."

[Figs. 1—8 are drawn from life, under the Zeiss 2·5 mm. (apert. 1·25) water immersion objective, compensating ocular 12. The remainder are from permanent preparations stained by Giemsa's method. Figs. 9—19 alcohol fixation, and Figs. 20—23 formalin fixation. Drawn under Zeiss 3 mm. apochromatic oil-immersion (1·40) with compensating ocular 18.]

Figs. 1—19.—Bacillus flexilis.

Fig. 1.—Ordinary vegetative individual, showing finely-granular, somewhat alveolar protoplasm, etc., and in which the outer layer is carefully focused.

Fig. 2.—An individual in which the spiral filament is very clearly seen.

Fig. 3.—Motile organism in which two fully-formed spores are seen.

Figs. 4—7, more enlarged drawings of one end, showing development of spore.

Fig. 4.—Beginning of spore formation.

Fig. 5.—Later stage.

Fig. 6.—Still later, formation of spore membrane.

Fig. 7.—Still later, fully formed spore.

Fig. 8.—A single spore, more highly magnified. A little cap-like projection is seen at one end.

Fig. 9.—Large, flattened specimen, showing chromidia, and faintly striated halo of pink cilia.

Fig. 10.—Division by constriction.

Fig. 11.—Subsequent stage in an organism which is about to sporulate. The constriction is disappearing, and large chromatin granules are arranging themselves in the first stage of a spiral.

Fig. 12.—A similar stage, probably somewhat earlier—less flattened.

Fig. 13.—At this stage the chromatin is all arranged in an irregular spiral.

Fig. 14.—The chromatin is now arranged in two large terminal masses, with an irregular intermediate portion.

Fig. 15.—A later stage, the intermediate part of spiral breaking up.

Fig. 16.—Still later. The terminal masses of chromatin are now definitely rounded off as the spores. They are stained red.

Fig. 17.—The spores are now stained a deep blue, owing to the presence of a newly-formed spore membrane.

Fig. 18.—The spores, as their membrane hardens, stain less deeply. They have moved away from the extremities, which are seen to be stained blue. The intermediate region, however, is now stained pink, owing to the presence of the broken up chromidia.

Fig. 19.—Spores now fully formed; remainder of cell breaking up.

Fig. 20.—Bacillus spirogyra. (*a*), Ordinary individual; (*b*), beginning of division; (*c*), organism which has just divided.

Fig. 21.—Spirillum monospora. (*a*), Normal individual; (*b*), individual in which the granules are collected at one end to form a spore; (*c*), spore now formed, provided with resistant membrane; (*d*), later stage, remains of cell breaking down and leaving the single spore.

Fig. 22.—Spirochæta bufonis. (*a*) and (*b*), two individuals, showing general structure.

Fig. 23.—Treponema-like organism from toad. (*a*), a thick individnal; (*b*), a very slender one. In (*c*) a tapering process (? flagellum) is seen at one end.